



Applicants: Linda B. Buck and Richard Axel  
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Please amend the subject application as follows:

In the specification:

Please replace the Brief Description of the Figures found on pages 6-12 of the specification with the amended Brief Description of the Figures as follows:

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Description of the Figures

Figure 1A-B. The Olfactory Neuroepithelium and a Pathway for Olfactory Signal Transduction.

(A). The Olfactory Neuroepithelium. The initial event in odor perception occurs in the nasal cavity in a specialized neuroepithelium which is diagramed here. Odors are believed to interact with specific receptors on the cilia of olfactory sensory neurons. The signal generated by these initial binding events are propagated by olfactory neuron axons to the olfactory bulb.

B<sup>1</sup> (B). A Pathway of Olfactory Signal Transduction. In this scheme, the binding of an odorant molecule to an odor-specific transmembrane receptor leads to the interaction of the receptor with a GTP-binding protein ( $G_{S[olf]}$ ). This interaction, in turn, leads to the release of the GTP-coupled  $\alpha$ -subunit of the G-protein, which then stimulates adenylyl cyclase to produce elevated levels of cAMP. The increase in cAMP opens nucleotide-gated cation channels, thus causing an alteration in membrane potential.

Figure 2A-B. A PCR Amplification Product Containing Multiple Species of DNA. cDNA prepared from olfactory epithelium RNA was

subjected to PCR amplification with a series of different primer oligonucleotides and the DNA products of appropriate size were isolated, further amplified by PCR, and size fractionated on agarose gels (A) (For details, see text). Each of these semipurified PCR products was digested with the restriction enzyme, Hinf I, and analyzed by agarose gel electrophoresis. Lanes marked "M" contain size markers of 23.1, 9.4, 5.6, 4.4, 2.3, 2.0, 1.35, 1.08, 0.87, 0.60, 0.31, 0.28, 0.23, 0.19, 0.12 and 0.07kb. (B). Twenty-two of the 64 PCR products that were isolated and digested with Hinf I are shown here. Digestion of one of these, PCR 13, yielded a large number of fragments whose sizes summed to a value much greater than that of the undigested PCR 13 DNA, indicating that PCR 13 might contain multiple species of DNA which are representatives of a multigene family.

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Figure 3. Northern Blot Analysis with a Mixture of Twenty Probes. One  $\mu$ g of polyA<sup>+</sup> RNA isolated from rat olfactory epithelium, brain, or spleen was size-fractionated in formaldehyde agarose, blotted onto a nylon membrane, and hybridized with a <sup>32</sup>P-labeled mixture of segments of 20 cDNA clones. The DNA segments were obtained by PCR using primers homologous to transmembrane domains 2 and 7.

Figure 4A-M. The Protein Sequences Encoded by Ten Divergent cDNA Clones. Ten divergent cDNA clones were subjected to DNA sequence analyses and the protein sequence encoded by each was determined (SEQ ID Nos: 71-80). Amino acid residues which are conserved in 60% or more of the proteins are shaded. The presence of seven hydrophobic domains (I-VII), as well as short conserved motifs

shared with other members of the superfamily, demonstrate that these proteins belong to the seven transmembrane (TM) domain protein superfamily. The transmembrane regions are indicated by labeled lines (I-VII) above the sequences. Motifs conserved among members of the family of olfactory proteins include those indicated by underlining below the sequences. In addition, the DRY motif C-terminal to TM3 is common to many members of the G-protein-coupled superfamily. However, all of the proteins shown here share sequence motifs not found in other members of this superfamily and are clearly members of a novel family of proteins.

Figure 5. Positions of Greatest Variability in the Olfactory Protein Family. In this diagram, the protein encoded by cDNA clone I15 (SEQ ID NO: 6 and SEQ ID NO: 80) is shown traversing the plasma membrane seven times with its N-terminus located extracellularly, and its C-terminus intracellularly. The vertical cylinders delineate the seven putative  $\alpha$ -helices spanning the membrane. Positions at which 60% or more of the 10 clones shown in Figure 4 share the same residue as I15 are shown as white balls. More variable residues are shown as black balls. The high degree of variability encountered in transmembrane domains III, IV, and V is evident in this schematic.

Figure 6A-D. The Presence of Subfamilies in a Divergent Multigene Family. Partial nucleotide sequences and deduced protein sequences were obtained for 18 different cDNA clones (SEQ ID Nos: 81-98). Transmembrane domain V along with the flanking loop sequences, including the entire cytoplasmic loop between transmembrane domains

V and VI, are shown here for each protein. Amino acid residues found in 60% or more of the clones in a given position are shaded (A). This region of the olfactory proteins (particularly transmembrane domain V) appears to be highly variable (see Figure 4). These proteins, however, can be grouped into subfamilies (B,C,D) in which the individual subfamily members share considerable homology in this divergent region of the protein.

Figure 7. Southern Blot Analyses with Non-crosshybridizing Fragments of Divergent cDNAs. Five  $\mu$ g of rat liver DNA was digested with Eco RI (A) or Hind III (B), electrophoresed in 0.75% agarose, blotted onto a nylon membrane, and hybridized to the  $^{32}$ P-labeled probes indicated. The probes used were PCR-generated fragments of: 1, clone F9 (identical to F12 in Figure 4); 2, F5; 3, F6; 4, I3; 5, I7; 6, I14; or 7, I15. The lane labeled "1-7" was hybridized to a mixture of the seven probes. The probes used showed either no crosshybridization or only trace crosshybridization with one another. The size markers on the left correspond to the four blots on the left (1-4) whereas the marker positions noted on the right correspond to the four blots on the right (5-7, "1-7").

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Figure 8. Northern Blot Analysis with a Mix of Seven Divergent Clones. One  $\mu$ g of polyA+ RNA from each of the tissues shown was size-fractionated, blotted onto a nylon membrane, and hybridized with a  $^{32}$ P-labeled mixture of segments of seven divergent cDNA clones (see Legend to Figure 7).

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Figure 9A-D. The nucleic acid and amino acid sequence of clone F3 (SEQ ID NO: 2 and SEQ ID NO: 71, respectively).

Figure 10A-D. The nucleic acid and amino acid sequence of clone F5 (SEQ ID NO: 3 and SEQ ID NO: 72, respectively).

Figure 11A-D. The nucleic acid and amino acid sequence of clone F6 (SEQ ID NO: 4 and SEQ ID NO: 73, respectively).

Figure 12A-D. The nucleic acid and amino acid sequence of clone F12 (SEQ ID NO: 1 and SEQ ID NO: 74, respectively).

Figure 13A-C. Partial nucleic acid and amino acid sequence of clone I3. Full nucleic acid and amino acid sequence of clone I3 are indicated in SEQ ID NO: 7 and SEQ ID NO: 75, respectively.

Figure 14A-D. The nucleic acid and amino acid sequence of clone I7 (SEQ ID NO: 8 and SEQ ID NO: 76, respectively).

Figure 15A-D. The nucleic acid and amino acid sequence of clone I8 (SEQ ID NO: 9 and SEQ ID NO: 77, respectively).

Figure 16A-D. The nucleic acid and amino acid sequence of clone I9 (SEQ ID NO: 10 and SEQ ID NO: 78, respectively).

Figure 17A-D. The nucleic acid and amino acid sequence of clone I14 (SEQ ID NO: 5 and SEQ ID NO: 79, respectively).

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Figure 18A-D. The nucleic acid and amino acid sequence of clone I15 (SEQ ID NO: 6 and SEQ ID NO: 80, respectively).

Figure 19A-D. The nucleic acid and amino acid sequence of human clone H5 (SEQ ID NO: 11 and SEQ ID NO: 12, respectively).

Figure 20A-C. The nucleic acid and amino acid sequence of clone J1, where the reading frame starts at nucleotide position 2 (SEQ ID NO: 13 and SEQ ID NO: 14, respectively).

Figure 21A-B. The nucleic acid and amino acid sequence of clone J2 (SEQ ID NO: 15 and SEQ ID NO: 16, respectively).

Figure 22A-B. The nucleic acid and amino acid sequence of clone J4, where the reading frame starts at nucleotide position 2 (SEQ ID NO: 17 and SEQ ID NO: 18, respectively).

Figure 23A-B. The nucleic acid and amino acid sequence of clone J7, where the reading frame starts at nucleotide position 2 (SEQ ID NO: 19 and SEQ ID NO: 20, respectively).

Figure 24A-B. The nucleic acid and amino acid sequence of clone J8, where the reading frame starts at nucleotide position 2 (SEQ ID NO: 21 and SEQ ID NO: 22, respectively).

Figure 25A-C. The nucleic acid and amino acid sequence of clone J11 (SEQ ID NO: 23 and SEQ ID NO: 24, respectively).

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Figure 26A-B. The nucleic acid and amino acid sequence of clone J14, where the reading frame starts at nucleotide position 2 (SEQ ID NO: 25 and SEQ ID NO: 26, respectively).

Figure 27A-B. The nucleic acid and amino acid sequence of clone J15, where the reading frame starts at nucleotide position 2 (SEQ ID NO: 27 and SEQ ID NO: 28, respectively).

Figure 28A-B. The nucleic acid and amino acid sequence of clone J16, where the reading frame starts at nucleotide position 2 (SEQ ID NO: 29 and SEQ ID NO: 30, respectively).

Figure 29A-B. The nucleic acid and amino acid sequence of clone J17, where the reading frame starts at nucleotide position 2 (SEQ ID NO: 31 and SEQ ID NO: 32, respectively).

Figure 30A-B. The nucleic acid and amino acid sequence of clone J19, where the reading frame starts at nucleotide position 2 (SEQ ID NO: 33 and SEQ ID NO: 34, respectively). The amino acid sequence after the stop codon is given in SEQ ID NO: 54.

Figure 31A-B. The nucleic acid and amino acid sequence of clone J20, where the reading frame starts at nucleotide position 2 (SEQ ID NO: 35 and SEQ ID NO: 36, respectively).

Figure 32. SOUTHERN BLOT: Five micrograms of DNA isolated from 1. Human placenta, 2. NCI-H-1011 neuroblastoma cells, or 3. CHP 134 neuroblastoma cells were treated with the restriction enzyme A. Eco

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